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Determination of biogenic amines in canned fish samples using head-space solid phase microextraction based on nanostructured polypyrrole fiber coupled to modified ionization region ion mobility spectrometry

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ABSTRACT

The head-space solid phase microextraction (HS-SPME) was applied to extraction and determination of histamine (HIS), putrescine (PUT), cadaverine (CAD), tyramine (TYR) in canned fish samples by ion mobility spectrometry (IMS) without any derivatization process. HIS and CAD have the same mobilities in nitrogen as buffer gas and their corresponding peaks are severely overlapped in ion mobility spectrum. Peak separation was acquired in the presence of 18-crown-6 vapor as complexation reagent into carrier gas and modified ionization region of IMS (MIR-IMS) at optimum flow rate. The interaction between 18-crown-6 and the mentioned amines forms nanocluster product ions with different cross section areas and ion mobilities. The effects of main extraction parameters on the efficiency of HS-SPME-MIR-IMS were investigated and optimized. Relative standard deviations (RSD%) of the biogenic amines determination at 50 $\mu g\,L^{-1}$ concentration level were obtained in range 5.7%–6.3%. Limits of detection for analytes were in the range of 0.6–1 $ng\,g^{-1}$. HS-SPME-MIR-IMS results indicate that the proposed method can be successfully used in biogenic amines analysis in water and food samples. Method validation was conducted by comparing our results with those obtained through GC–MS method.

1. Introduction

Biogenic amines are simple nitrogen compounds that are produced by decarboxylation of free amino acids during the degradation processes of proteins [1]. Histamine (HIS), putrescine (PUT), cadaverine (CAD), tyramine (TYR), β – phenyl ethylamine (β-EPA), spermine (SPM) and spermidine (SPD) are considered as the most important biogenic amines which are found in food [2]. Biogenic amines in high concentration become toxic and lead to a wide range of symptoms as palpitations, nausea and headache. In addition, they are precursors of carcinogenic N-nitroso compounds [1,3]. Analysis of biogenic amines is important because of their toxicity and their usage as an indicating parameter to food quality [4]. Different methods have been used to analyze biogenic amines in food samples. Common methods are high-performance liquid chromatography (HPLC) [5–8], gas chromatography-flame ionization detection (GC-FID) [9], gas chromatography-mass spectrometry (GC–MS) [10,11], and capillary electrophoresis (CE) [12–14]. The disadvantage of these techniques is sample preparation, which usually requires derivatization of the biogenic amines to improve separation and reduce tailing [1,15]. Due to low volatility and lack of chromophores of most BA derivatization procedure has been usually applied [14]. A fast technique for determination of biogenic amines is ion mobility spectrometry (IMS). IMS with ^{63}Ni as the ionization source and n-Nonylamine as the reagent gas have been used by Karpas et al. for the analysis of biogenic amines in vaginal fluid and in meat food products for the first time [16,17].

Ion mobility spectrometry is an analytical technique that separates gas-phase ions based on differences in their mobilities through a gaseous atmosphere under the influence of a uniform electric field. This technique has been used for the detection of a variety of compounds due to its excellent sensitivity and quick operation [18]. One disadvantage of IMS is low resolving power which causes overlapping peaks in ion mobility spectrum [19]. To cope with this problem, gas phase modifiers are introduced into the buffer gas of mobility spectrometers to improve the resolution of mixtures. These modifiers are polar volatile compounds that interact with the analyte ions to form ion clusters. These

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clusters have different shapes and sizes and spend different time in the drift region [20]. Several studies have been performed in order to evaluate the effect of gas phase modifier on mobility of analytes and peak separation [19,21–23]. Recently, many studies have performed based on the use of selective complexation reagents for improving separations. Cyclic polyether and acyclic polyether were used in IMS-MS analyses of amines [24], amino acids [25] and peptides [25,26]. Crown ethers are interesting due to the existence substantial understanding of the interactions of these molecules with different species such as ammonium, amines and peptides [27–29].

The objective of the present work is to eliminate the overlap of biogenic amine peaks and determination of them in food samples without derivatization steps. We tested the introduction of 18-crown-6 in the carrier gas in order to modification of ionization region of IMS (MIR-IMS). The effect of modifier flow rate, temperature and electric field on the separation of peaks was investigated. Due to the complex matrix in food products, we used head space solid phase microextraction as the sample preparation. In this study, a dodecylbenzenesulfonate-doped nanostructured polypyrrole coating was used as a fiber for HS-SPME. The effect of more important extraction parameters on extraction efficiency including pH, salt effect, extraction time and temperature was also studied and optimized.

2. Experimental

2.1. Reagent and chemical

The biogenic amines including histamine (HIS), cadaverine (CAD), tyramine (TYR) and putrescine (PUT) hydrochloride were purchased (≥99%) from Sigma–Aldrich (Steinheim, Germany). Pyrrole was obtained from Fluka (Buchs, Switzerland) and distillated before use. Macrocyclic polyether 18-crown-6, Sodium dodecylbenzenelsulfonate (SDBS), sodium hydroxide, trichloroacetic acid, methanol, hydrochloric acid (37%) and sodium chloride (analytical grade) were obtained from Merck (Darmstadt, Germany). The internal standard (IS), 1, 7-diaminoheptane, was obtained from Aria Kimia Gostar Co (Tehran, Iran). Isobutyl chloroformate (IBCF) was purchased from Sigma (St Louis, MO, USA). Canned fish samples were purchased from local supermarkets (Tehran, Iran). Stock standard solutions of HIS, CAD, PUT and TYR were prepared at a concentration of 2000 $\mu g\,mL^{-1}$ in HCl (0.1 $mol\,L^{-1}$). Mixed standard solution was prepared at concentration of 100 $\mu g\,mL^{-1}$ in double distilled water. Working solutions (3–250 $ng\,mL^{-1}$) were prepared by diluting stock solutions with double distilled water. The stock solutions were refrigerated at 4 °C. All solvents were analytical reagent grade or HPLC grade. The pH of the solutions was adjusted by HCl and sodium hydroxide.

2.2. Instrumentation

In these experiments an IMS with a corona ionization source was used to determination and quantification biogenic amines. The IMS instrument used in the present study was manufactured by TOF Tech. Pars Company at Isfahan University of Technology, Iran. Due to the high proton affinity of biogenic amines, positive mode was chosen for instrument operation. The optimized experimental conditions for obtaining the ion mobility spectra of the compounds are listed in Table 1.

Table 1
The optimized experimental ion mobility spectrometry (IMS) conditions.

Parameter	Value
Corona current	6 µA
Drift voltage	7000 V
Drift gas flow, N_2	450 $mL^{-1}\,min^{-1}$
Carrier gas flow, N_2	150 $mL^{-1}\,min^{-1}$
Cell temperature	200 °C
Injector temperature	220 °C
Drift tube length	11 cm
Shutter grid	150 µs
Modifier flow rare	40 $mL\,min^{-1}$

GC–MS analyses were carried out by 7890A GC system from Agilent Technologies (Palo Alto, CA, USA) equipped with a triple-axis detector and coupled with a 5975C inert MSD network mass selective detector. The chemical compounds were separated on HP-5 MS capillary column (30 m × 0.25 mm I.D., 0.25 µm film thickness). A split/splitless injector was used in a split mode with a split ratio of 1:50. Helium was the carrier gas in a constant flow of 1 $mL\,min^{-1}$. The temperature program commenced at 190 °C for 5 min, and was then raised by 7 $°C\,min^{-1}$–250 °C, held for 1 min. Finally, the temperature was increased rapidly by 20 $°C\,min^{-1}$–300 °C and kept for 10 min. The injector temperature and the auxiliary temperature were set at 280 °C. The analysis was performed using the selected ion monitoring (SIM) mode, and each analyte was quantified using one qualifier ion. Ions monitored were as follows: m/z 170 for PUT, m/z 130 for CAD, m/z 194 for HIA, m/z 107 for TYA and m/z 112 for IS. Centrifuging (Heltich Rotorfix 32A) was applied at 4000 rpm (2700 g) to separate the mixture.

The SPME holder for manual sampling was obtained from Azar Electrode (Ourumieh, Iran). Electrochemical polymerization of pyrrole was carried out by means of AUTOLAB (PGSTAT 30 model) potentiostat. An electrochemical cell including a platinum. (Pt) working electrode (2 a cm × 300 µm O.D.), Pt counter electrode and a double junction saturated calomel (dj-SCE) electrode, as reference electrode was used for preparation of the polymer.

The PPy film doped with DBS as the solid phase (PPy-DBS) was prepared electrochemically using a three-electrode system. PPy-DBS film was directly electrodeposited on the surface of a platinum wire (as the working electrode) from an aqueous solution containing pyrrole (0.1 $mol\,L^{-1}$) and SDBS (7×10^{-3} $mol\,L^{-1}$) by the application of a constant potential of +1.1 V versus the Ag/AgCl electrode for 300 s. Prior to each electrochemical polymerization, the synthesis solution was deoxygenated by purging it with nitrogen for 10 min and the working electrode was cleaned in methanol and water using an ultrasonic bath for 10 min, respectively. Finally, it was dried at room temprature before performing the experiments. The electrochemical coating has a porous structure. The deposited polymers are spherical with a particle size between 90 and 300 nm. The thickness of the film was estimated by comparing the scratching film with the substrata surface and was about 50 µm according to the micrograph [30].

2.3. Modifier introduction

An online system was required to introduce 18-crown-6 vapor into the carrier gas flow. To achieve this, first 100 mg of solid 18-crown-6 was placed in a vessel of 10 cm^3 and was connected to the carrier gas via a capillary tube whereas nitrogen gas was flowing into the vessel at a flow rate of 40 $mL\,min^{-1}$. The temperature was maintained by placing the vessel in a water-bath heated to 35 °C. The solid 18-crown-6 is in equilibrium with the 18-crown-6 vapor. The vapor of modifier was diluted with nitrogen gas and injected into the flowing carrier gas. This method ensures continuous introduction of a constant amount of modifier into the reaction region. Initial experiments to assess the ability of 18-crown-6 modifier in peak separation of biogenic amines were performed by a fine nichrome filament (as probe) that hold on a brass cap is used to introduce sample. The 2 µL of aqueous solutions of biogenic amines

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